

CLAIMS

1. A method for making a composition of magnetic nanoparticles which includes the step of forming said magnetic nanoparticles, each within a protein template, wherein a liquid composition of said protein templates or subunits thereof is subjected to a microporous membrane filtration step prior to formation of said magnetic nanoparticles.
2. A method in accordance with claim 1, wherein said protein template is selected from the group comprising flagellar L-P rings, microtubules, bacteriophages, chaperonins, virus capsids and members of the ferritin family.
3. A method in accordance with claim 2, wherein said protein template comprises a member of the ferritin family.
4. A method in accordance with claim 3, wherein said member of the ferritin family is selected from DPS and apoferritin.
5. A method in accordance with claim 4, wherein said member of the ferritin family is apoferritin.
6. A method in accordance with any preceding claim, wherein said magnetic nanoparticles comprise ferri- or ferro-magnetic metals, metal alloys, M-type or spinel ferrite.
7. A method in accordance with claim 6, wherein said ferri- or ferro-magnetic metal is selected from the group comprising cobalt, iron or nickel.
8. A method in accordance with claim 6, wherein said metal or metal alloy is selected from the group comprising aluminium, barium, bismuth, cerium, chromium, cobalt, copper, dysprosium, erbium, europium, gadolinium, holmium, iron, lanthanum, lutetium, manganese, molybdenum, neodymium, nickel, niobium, palladium, platinum, praseodymium, promethium, samarium, strontium, terbium, thulium, titanium, vanadium, ytterbium, and yttrium or a mixture thereof.
9. A method in accordance with claim 6, wherein said alloy is a binary alloy or a ternary alloy.

10. A method in accordance with claim 9, wherein said binary alloy is selected from the group comprising cobalt-nickel, iron-platinum, cobalt-palladium, iron-palladium, samarium-cobalt.
- 5 11. A method in accordance with claim 9, wherein said ternary alloy is selected from the group comprising dysprosium-iron-turbide or neodymium-iron boride, iron-cobalt-platinum, cobalt-nickel platinum, or cobalt-nickel-chromium.
12. A method in accordance with any preceding claim wherein said nanoparticles comprise cobalt or platinum or alloys thereof.
- 10 13. A method in accordance with claim 12 wherein said nanoparticles comprise an alloy of cobalt and platinum.
14. A method in accordance with any preceding claim, wherein said liquid composition is an aqueous solution.
- 15 15. A method in accordance with any preceding claim, wherein the pore size of the membrane filter is in the range from about 0.02-10 $\mu$ m.
16. A method in accordance with any preceding claim, wherein the pore size of the membrane filter is less than about 1 $\mu$ m.
17. A method in accordance with claim 16, wherein the pore size of the membrane filter is less than about 0.5 $\mu$ m.
- 20 18. A method in accordance with claim 17, wherein the pore size of the membrane filter is less than about 0.2 $\mu$ m.
19. A method in accordance with claim 18, wherein the pore size of the membrane filter is about 0.1 $\mu$ m.
- 25 20. A method in accordance with any preceding claim wherein the membrane filter is a disc filter.
21. A method in accordance with any preceding claim, wherein the membrane filter is made from a material selected from the group comprising polymeric materials, metals, ceramics, glass or carbon.
- 30 22. A method in accordance with claim 21, wherein said material comprises a polymer.
23. A method in accordance with claim 22, wherein said polymer is selected from the group comprising polysulphones, polyethersulphones (PES), polyacrylates, polyvinylidenes, polytetrafluoroethylene (PTFE), cellulose, cellulose esters or co-polymers thereof.
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24. A method in accordance with claim 23, wherein said polymer is polyethersulphone or a polyvinylidene.
25. A method in accordance with any preceding claim, wherein said protein is present in said liquid composition at a concentration in the range from about 10-50mg/ml.
26. A method in accordance with any preceding claim, wherein the pH of said liquid composition is in the range from about 5.0-7.0.
27. A method in accordance with any preceding claim, wherein the liquid composition is subjected to an applied positive pressure during the filtration step.
28. A method in accordance with any preceding claim, wherein said magnetic nanoparticles have a diameter (or largest diameter in the case of non-spheroidal particles) not greater than about 100nm.
29. A method in accordance with claim 28, wherein said magnetic nanoparticles have a diameter not greater than about 50nm.
30. A method in accordance with claim 29, wherein said magnetic nanoparticles have a diameter of about 20nm or less.
31. A method in accordance with any preceding claim, wherein said magnetic nanoparticles vary in their largest dimension by no more than about 20%.
32. A method in accordance with claim 31, wherein said magnetic nanoparticles vary in their largest dimension by no more than about 10%.
33. A method in accordance with claim 32, wherein said magnetic nanoparticles vary in their largest dimension by no more than about 5%.
34. A method in accordance with any preceding claim, wherein the formation step of said magnetic nanoparticles includes the incremental addition of sources of ions of the metal or metals to comprise or consist said magnetic nanoparticles.
35. A method in accordance with claim 34, wherein said cation and anion sources are added in sufficient amounts to provide 1-200 atoms of the cation and anion per encapsulating particle per iteration.
36. A method in accordance with claim 35, wherein said cation and anion sources are added in sufficient amounts to provide 20-100

atoms of the cation and anion per encapsulating particle per iteration.

- 5 37. A method in accordance with claim 36, wherein said cation and anion sources are added in sufficient amounts to provide about 50 atoms of the cation and anion per encapsulating particle per iteration.
38. A method in accordance with any preceding claim, wherein the source of the metal ions is a salt of the metal or metals.
- 10 39. A method in accordance with claim 38 wherein said salt is tetrachloroammoniumplatinite.
40. A method in accordance with any preceding claim, wherein the formation of said magnetic nanoparticles takes place under an inert atmosphere.
- 15 41. A method in accordance with any preceding claim, wherein the formation of said magnetic nanoparticles takes place at a temperature of at least about 24°C.
42. A method in accordance with claim 41, wherein the formation of said magnetic nanoparticles takes place at a temperature in the range from about 25°C to about 60°C.
- 20 43. A method in accordance with claim 42, wherein the formation of said magnetic nanoparticles takes place at a temperature in the range from about 35°C to about 50°C.
44. A method in accordance with any preceding claim, wherein the method further comprises a magnetic fractionation step.
- 25 45. A method in accordance with claim 44, wherein said magnetic fractionation step comprises passing said liquid composition through a column comprising magnetic powder at a flow rate in the range from about 0.2-10ml/min<sup>-1</sup>.
- 30 46. A method in accordance with any preceding claim, wherein the encapsulating shell is functionalised with a ligand selected from the group comprising biotin, avidin, an antibody or derivative thereof, a receptor molecule, an opsonin or a metal binding ligand.
47. A method in accordance with any preceding claim, wherein the encapsulating shell is removed or treated.
- 35 48. A method in accordance with claim 47, wherein said encapsulating shell is removed by enzymatic degradation or pH denaturation.

49. A method in accordance with claim 48, wherein the enzyme used to remove the protein is a protease.
50. A method in accordance with claim 48, wherein the pH denaturation is effected by adjusting the pH of the composition to a value below about 4.0 or above about 9.0.
51. A method in accordance with claim 47, wherein the encapsulating shell is carbonised.
52. A method for treating a liquid composition of magnetic nanoparticles, each formed within a macromolecular template, wherein said method includes the step of subjecting said composition to a microporous membrane filtration step.
53. A method in accordance with claim 52, wherein said macromolecular template comprises organic or inorganic material.
54. A method in accordance with claim 53, wherein said inorganic material is selected from the group comprising siloxanes, silanes or derivatives thereof.
55. A method in accordance with claim 53, wherein said organic material is an organic macromolecule.
56. A method in accordance with claim 55, wherein said organic macromolecule is selected from the group comprising surfactants, polymers and proteins.
57. A method in accordance with claim 56, wherein said protein is selected from the group comprising flagellar L-P rings, microtubules, bacteriophages, chaperonins, virus capsids and members of the ferritin family.
58. A method in accordance with claim 57, wherein said protein template comprises a member of the ferritin family.
59. A method in accordance with claim 58, wherein said member of the ferritin family is selected from the group comprising DPS and apoferritin.
60. A method in accordance with claim 59, wherein said member of the ferritin family is apoferritin.
61. A method in accordance with any of claims 52-60, wherein said magnetic nanoparticles comprise ferri- or ferro-magnetic metals, metal alloys, M-type or spinel ferrite.

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62. A method in accordance with claim 61, wherein said ferri- or ferro-magnetic metal is selected from the group comprising cobalt, iron or nickel.
63. A method in accordance with claim 61, wherein said metal or metal alloy is selected from the group comprising aluminium, barium, bismuth, cerium, chromium, cobalt, copper, dysprosium, erbium, europium, gadolinium, holmium, iron, lanthanum, lutetium, manganese, molybdenum, neodymium, nickel, niobium, palladium, platinum, praseodymium, promethium, samarium, strontium, terbium, thulium, titanium, vanadium, ytterbium, and yttrium or a mixture thereof.
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64. A method in accordance with claim 61, wherein said alloy is a binary alloy or a ternary alloy.
65. A method in accordance with claim 64, wherein said binary alloy is selected from the group comprising cobalt-nickel, iron-platinum, cobalt-palladium, iron-palladium, samarium-cobalt.
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66. A method in accordance with claim 64, wherein said ternary alloy is selected from the group comprising dysprosium-iron-turbide or neodymium-iron boride, iron-cobalt-platinum, cobalt-nickel platinum, or cobalt-nickel-chromium.
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67. A method in accordance with any of claims 52-66 wherein said nanoparticles comprise cobalt or platinum or alloys thereof.
68. A method in accordance with claim 67 wherein said nanoparticles comprise an alloy of cobalt and platinum.
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69. A method in accordance with any of claims 52-69, wherein said liquid composition is an aqueous solution.
70. A method in accordance with any of claims 52-69, wherein the pore size of the membrane filter is in the range from about 0.02-10 $\mu$ m.
71. A method in accordance with any of claims 52-70, wherein the pore size of the membrane filter is less than about 1 $\mu$ m.
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72. A method in accordance with claim 71, wherein the pore size of the membrane filter is less than about 0.5 $\mu$ m.
73. A method in accordance with claim 72, wherein the pore size of the membrane filter is less than about 0.2 $\mu$ m.

74. A method in accordance with claim 73, wherein the pore size of the membrane filter is about 0.1 $\mu$ m.
75. A method in accordance with any of claims 52-74 wherein the membrane filter is a disc filter.
- 5 76. A method in accordance with any of claims 52-75, wherein the membrane filter is made from a material selected from the group comprising polymeric materials, metals, ceramics, glass or carbon.
77. A method in accordance with claim 76, wherein said material comprises a polymer.
- 10 78. A method in accordance with claim 77, wherein said polymer is selected from the group comprising polysulphones, polyethersulphones (PES), polyacrylates, polyvinylidenes, polytetrafluoroethylene (PTFE), cellulose, cellulose esters or co-polymers thereof.
- 15 79. A method in accordance with claim 78, wherein said polymer is polyethersulphone or a polyvinylidene.
80. A method in accordance with any of claims 56-79, wherein said protein is present in said composition at a concentration in the range from about 0.1-20mg/ml.
- 20 81. A method in accordance with any of claims 52-80, wherein the pH of the composition is in the range from 7-8.5.
82. A method in accordance with any of claims 52-81, wherein the composition is subjected to an applied positive pressure during the filtration step.
- 25 83. A method in accordance with any of claims 52-84, wherein said magnetic nanoparticles have a diameter (or largest diameter in the case of non-spheroidal particles) not greater than about 100nm.
84. A method in accordance with claim 83, wherein said magnetic nanoparticles have a diameter (or largest diameter in the case of
- 30 non-spheroidal particles) not greater than about 50nm.
85. A method in accordance with claim 84, wherein said magnetic nanoparticles have a diameter (or largest diameter in the case of non-spheroidal particles) of about 20nm or less.
- 35 86. A method in accordance with any of claims 50-85, wherein said magnetic nanoparticles vary in their largest dimension by no more than about 20%.

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87. A method in accordance with claim 86, wherein said magnetic nanoparticles vary in their largest dimension by no more than about 10%.
88. A method in accordance with claim 87, wherein said magnetic nanoparticles vary in their largest dimension by no more than about 5%.
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89. A method in accordance with any of claims 50-88, wherein the method further comprises a magnetic fractionation step.
90. A method in accordance with claim 89, wherein said magnetic fractionation step comprises passing the composition through a column comprising magnetic powder at a flow rate in the range from about 0.2-10ml/min<sup>-1</sup>.
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91. A method in accordance with claim 89 or 90, wherein the magnetic filtration step is performed prior to the membrane filtration step.
92. A method in accordance with any of claims 50-91, wherein the encapsulating shell is functionalised with a ligand selected from the group comprising biotin, avidin, an antibody or derivative thereof, a receptor molecule, an opsonin or a metal binding ligand.
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93. A method in accordance with any of claims 50-92, wherein the encapsulating shell is removed or treated.
94. A method in accordance with claim 93, wherein said encapsulating shell is removed by enzymatic degradation or pH denaturation.
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95. A method in accordance with claim 94, wherein the enzyme used to remove the encapsulating shell is a protease.
96. A method in accordance with claim 94, wherein the pH denaturation is effected by adjusting the pH of the composition to a value below about 4.0 or above about 9.0.
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97. A method in accordance with claim 93, wherein the encapsulating shell is carbonised.
98. A stable composition of magnetic nanoparticles wherein each nanoparticle is encapsulated by an encapsulating material, wherein at least 70% by weight of the nanoparticles are not in an agglomerated form and wherein the composition comprises no more than 30% free encapsulating material, based on the total weight of the encapsulating material in the composition.
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99. A composition in accordance with claim 98, wherein at least 80% by weight of said nanoparticles are not agglomerated.
100. A composition in accordance with claim 99, wherein at least 90% by weight of said nanoparticles are not agglomerated.
- 5 101. A composition in accordance with any of claims 98-100, wherein said encapsulating material comprises organic or inorganic material.
102. A composition in accordance with claim 101, wherein said inorganic material is selected from the group comprising siloxanes, silanes or derivatives thereof.
- 10 103. A composition in accordance with claim 102, wherein said organic material comprises an organic macromolecule.
104. A composition in accordance with claim 103, wherein said organic macromolecule is selected from the group comprising surfactants, polymers and proteins.
- 15 105. A composition in accordance with claim 104, wherein said protein is selected from the group comprising flagellar L-P rings, microtubules, bacteriophages, chaperonins, virus capsids and members of the ferritin family.
- 20 106. A composition in accordance with claim 105, wherein said protein encapsulating material comprises a member of the ferritin family.
107. A composition in accordance with claim 106, wherein said member of the ferritin family is DPS or apoferritin.
108. A composition in accordance with claim 107, wherein said member of the ferritin family is apoferritin.
- 25 109. A composition in accordance with any of claims 98-108, wherein said magnetic nanoparticles comprise ferri- or ferro-magnetic metals, metal alloys, M-type or spinel ferrite.
110. A composition in accordance with claim 109, wherein said ferri- or ferro-magnetic metal is selected from the group comprising cobalt, iron or nickel.
- 30 111. A composition in accordance with claim 109, wherein said metal or metal alloy is selected from the group comprising aluminium, barium, bismuth, cerium, chromium, cobalt, copper, dysprosium, erbium, europium, gadolinium, holmium, iron, lanthanum, lutetium, manganese, molybdenum, neodymium, nickel, niobium, palladium, platinum, praseodymium, promethium, samarium, strontium,
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terbium, thulium, titanium, vanadium, ytterbium, and yttrium or a mixture thereof.

112. A composition in accordance with claim 109, wherein said alloy is a binary alloy or a ternary alloy.

5 113. A composition in accordance with claim 112, wherein said binary alloy is selected from the group comprising cobalt-nickel, iron-platinum, cobalt-palladium, iron-palladium, samarium-cobalt.

10 114. A composition in accordance with claim 112, wherein said ternary alloy is selected from the group comprising dysprosium-iron-turbide or neodymium-iron boride, iron-cobalt-platinum, cobalt-nickel platinum, or cobalt-nickel-chromium.

115. A composition in accordance with any of claims 98-114 wherein said nanoparticles comprise cobalt or platinum or alloys thereof.

15 116. A composition in accordance with claim 115 wherein said nanoparticles comprise an alloy of cobalt and platinum.

117. A composition in accordance with any of claim 98-116, wherein said composition is a liquid composition.

118. A composition in accordance with claim 117, wherein said liquid composition is an aqueous solution.

20 119. A composition in accordance with any of claims 117 or 118, wherein the pH of the composition is in the range from about 5.0-7.0.

120. A composition in accordance with any of claims 98-119, wherein said magnetic nanoparticles have a diameter (or largest diameter in the case of non-spheroidal particles) not greater than about 100nm.

25 121. A composition in accordance with claim 120, wherein said magnetic nanoparticles have a diameter not greater than about 50nm.

122. A composition in accordance with claim 121, wherein said magnetic nanoparticles have a diameter of about 20nm or less.

30 123. A composition in accordance with any of claims 98-122, wherein said magnetic nanoparticles vary in their largest dimension by no more than about 20%.

124. A composition in accordance with claim 123, wherein said magnetic nanoparticles vary in their largest dimension by no more than about 10%.

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125. A composition in accordance with claim 124, wherein said magnetic nanoparticles vary in their largest dimension by no more than about 5%.
126. A composition in accordance with any of claims 98-125, wherein the encapsulating shell is functionalised with a ligand selected from the group comprising biotin, avidin, an antibody or derivative thereof, a receptor molecule, an opsonin or a metal binding ligand.
127. A composition in accordance with any of claims 98-126, wherein the encapsulating shell is removed or treated.
- 10 128. A composition in accordance with claim 127, wherein said encapsulating shell is removed by enzymatic degradation or pH denaturation.
129. A composition in accordance with claim 128, wherein the enzyme used to remove the protein is a protease.
- 15 130. A composition in accordance with claim 128, wherein the pH denaturation is effected by adjusting the pH of the composition to a value below about 4.0 or above about 9.0.
131. A composition in accordance with claim 130, wherein the encapsulating shell is carbonised.
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